

What is claimed is:

1. A computer based method for preparing a stem cell factor (SCF) analog comprising the steps of:
  - (a) providing computer expression of the three dimensional structure of an SCF molecule using its crystal structure;
  - (b) selecting from the computer expression of step (a) at least one site on the SCF molecule for alteration;
  - (c) preparing a SCF molecule having an alteration at said at least one selected site; and
  - (d) optionally, testing the SCF molecule for a desired characteristic.
2. The method of claim 1, wherein the SCF analog comprises a polypeptide having an amino acid sequence portion of SCF capable of binding a receptor and having the overall three-dimensional conformation as shown in Figures 2A-2B, wherein the three-dimensional conformation is:
  - a) anti-parallel, double-cross over 4-alpha helical bundle with a left hand twist; and
  - b) overall dimensions of approximately 85 Å x 30 Å x 20 Å.
3. The method of claim 1, wherein the SCF analog

comprises electron density distributions as set forth in Figures 1A, 1B, and 1C.

4. The method of claim 1 wherein the SCF molecule is a native SCF or a selenomethionyl SCF.

5. The method of claim 1 wherein the site on the SCF molecule for alteration is a receptor binding site on the surface of the SCF molecule or a non-receptor site of the SCF.

*See C20* 6. The method of claim 5, wherein the receptor binding site comprises approximately amino acid residues 79-95.

7. An isolated SCF analog prepared according to the method of claim 1.

8. The isolated SCF analog of claim 7, wherein the SCF analog comprises a polypeptide having an amino acid sequence portion of SCF capable of binding a receptor and having the overall three-dimensional conformation as shown in Figures 2A-2B, wherein the three-dimensional conformation is:

a) anti-parallel, double-cross over 4-alpha helical bundle with a left hand twist; and

b) overall dimensions of approximately 85 Å x 30 Å x 20 Å..

9. The isolated SCF analog of claim 10, wherein the SCF analog comprises electron density distributions altered from those set forth in Figures 1A, 1B, and 1C.
10. A composition comprising an isolated SCF analog prepared according to the method of claim 1 effective to treat a subject and a pharmaceutically acceptable carrier.
11. A method of treating a subject having a disorder requiring SCF comprising administration of a composition comprising an isolated SCF analog prepared by the method of claim 1 or a compound designed by the method of claim 32.
12. The method of claim 11, wherein the subject has a blood disorder.
13. The method of claim 12, wherein the disorder which the subject has is anemia, myeloproliferative disorder, neoplasia, nerve damage, infertility, intestinal damage, a pigmentation disorder, or immunodeficiency.
14. The method of claim 11, wherein the administration of the isolated SCF analog is for ex vivo or in vivo production of peripheral blood progenitors, ex vivo or in vivo stem cell expansion, ex vivo or in vitro

growth of epithelial cells, ex vivo or in vitro growth of stromal cells, ex vivo or in vitro dendritic cell stimulation, and in vivo cell mobilization.

Sub  
C21

15. A method for designing a compound capable of binding to the stem cell factor (SCF) receptor site of comprising the steps of:

- a) determining a binding site for the SCF receptor on the SCF based on the three-dimensional structure of SCF or an SCF polypeptide or portion/fragment thereof, atomic coordinates computed from X-ray diffraction data of a crystal comprising a polypeptide having an amino acid sequence portion of SCF capable of binding the receptor; and
- b) designing a compound comprising an entity that binds the SCF receptor.

16. The method of claim 15, wherein the design of the compound of step (b) is determined by shape complementarity or by estimated interaction energy.

17. The method of claim 15, wherein the designed compound fits an SCF receptor binding site on SCF receptor as shown in Figure 6.

18. The method of claim 15, wherein the designed compound fits an SCF receptor binding site on

SCF receptor as shown in Figures 7A or 7B.

19. The method of claim 15, wherein the designed compound is a double-headed SCF ligand analog having the structure set forth in Figure 10A.

20. The method of claim 19, wherein each ligand head of the double-headed SCF ligand analog is an oligopeptide having the structure set forth in Figure 10B.

Sub  
C22

21. The method of claim 20, wherein the oligopeptide comprises a sequence, wherein functional moiety  $F_1$  corresponds to a segment of amino acid residues from within N-terminal residues 1-10 of SCF, functional moiety  $F_2$  corresponds to a segment of amino acid residues from within residues 79-95 of SCF, and functional moiety  $F_3$  corresponds to a segment of amino acid residues located within three amino acid residues of amino acid residue 127, wherein  $F_1$ ,  $F_2$ , and  $F_3$  are connected by connecting peptide segments  $X_n$ ,  $X_m$ , and  $X_p$ , respectively, wherein  $n=0-5$ ,  $m=0-5$  and  $p=3-8$  amino acid residues, respectively, and the conjugation moiety  $F_L$  is a cysteine residue.

22. The method of claim 21, wherein the functional moieties  $F_1$ ,  $F_2$ , and  $F_3$  on the ligand heads have been selected by bacterial phage display

Sub  
D2

for optimal receptor binding.

23. The method of claim 21, wherein the functional moieties and connecting peptide segments of an active oligopeptide ligand head are replaced by chemical mimetics.

Sub  
C23

24. The method of claim 15, wherein an appropriate chemical scaffold of connecting segments has been designed to comprise (present) functional moieties  $F_1$ ,  $F_2$ , and  $F_3$ , which have been selected by combinatorial chemistry for optimal receptor binding from a library of chemical moieties complementary to receptor-binding sites on the surface of SCF.

25. The method of claim 15, wherein the linker comprises an organic polymer having two ends capped at each end by a reactive capping moiety,  $F_c$ , which react covalently with the conjugation moiety,  $F_L$ , on the ligand head.

Sub  
D3

26. The method of claim 25, wherein the organic polymer is polyethyleneglycol (PEG) comprising the structure  $H[OCH_2CH_2]_nOH$ , wherein  $n$  is 10-20.

27. The method of claim 25, wherein the capping moiety,  $F_c$ , is a thiol-reactive group such as N-ethyl maleimide.

28. The method of claim 15, wherein the conjugating moiety, F<sub>L</sub>, is a thiol containing group such as cysteine.
29. A compound designed by the method of claim 15.
30. A composition comprising the compound designed by the method of claim 15 and a pharmaceutically acceptable carrier.
31. The compound of claim 30, wherein the compound comprises an isolated SCF analog, whose alteration site is a receptor-binding site on the surface of the altered SCF molecule.
32. A method of treating a subject comprising administration of a compound designed by the method of claim 32.
33. The method of claim 32, wherein the subject has a blood disorder.
34. The method of claim 33, wherein the blood disorder is anemia or immunodeficiency.
35. The method of claim 32, wherein the compound is an isolated SCF analog.
36. A method of stimulating the production of hematopoietic cells in a subject comprising administering an isolated stem cell factor

(SCF) analog.

37. The method of claim 36, wherein isolated stem cell factor (SCF) analog is prepared by the method of claim 1 or designed by the method of claim 32.

Sub  
C24

38. The method of claim 37, wherein the isolated SCF analog comprises amino acid residues of native or recombinant SCF1-165 or amino acid residues of a recombinant selenomethionyl SCF1-141.

Sub  
C25

39. An isolated stem cell factor (SCF) molecule, which is an altered SCF, comprising any portion of amino acids 1-165 of a human SCF polypeptide, optionally comprising an N-terminal methionine before amino acid residue 1, wherein the polypeptide has an amino acid sequence portion of SCF capable of binding to the SCF receptor.

Sub  
C26

40. The altered isolated stem cell factor molecule of claim 39, wherein an alteration is selected from the group consisting of deletion, insertion and substitution of at least one amino acid residue from the naturally occurring amino acid sequence of SCF.

Sub  
C27

41. The altered isolated stem cell factor molecule of claim 40, wherein an alteration is a



Truncated SCF comprising amino acids 1-141 of a human SCF polypeptide, optionally comprising an N-terminal methionine before amino acid residue 1.

Sub  
C27  
ended

5

Sub  
C28

42. The altered isolated stem cell factor molecule of claim 40, wherein the substitution of at least one amino acid residue is selected from the group consisting of SCF(Y26C) disulfide-linked dimer, SCF(D25C), SCF(K62C), SCF(K78N, N81K), SCF(R117A, I118A), SCF(E92A, S95A), and SCF(D124A, K127D).

43. A stem cell factor molecule of claim 40, wherein the overall three-dimensional conformation of the stem cell factor molecule has an altered three-dimensional structure of the  $\alpha$ C- $\beta$ 2 loop.

44. A pharmaceutical composition comprising the altered isolated SCF molecule of claim 39 and a pharmaceutically acceptable carrier.

45. A stem cell factor molecule of claim 39, wherein the molecule is a hybrid molecule of the altered stem cell factor molecule and a second protein or fragment thereof.

46. A stem cell factor molecule of claim 39, wherein the alteration of the  $\alpha$ C- $\beta$ 2 loop is a change in length of the amino acid sequence of

10

15

20

25

30

the  $\alpha$ C- $\beta$ 2 loop by a deletion or an insertion of at least one amino acid residue or a change in at least one amino acid residue from the naturally occurring amino acid residue(s) of the  $\alpha$ C- $\beta$ 2 loop.

Sub  
C29

47. The altered isolated stem cell factor molecule of claim 46, wherein the change in said at least one amino acid residue from the naturally occurring amino acid residue(s) is selected from the group consisting of SCF(Y26C) disulfide-linked dimer, SCF(D25C), SCF(K62C), SCF(K78N, N81K), SCF(R117A, I118A), SCF(E92A, S95A), and SCF(D124A, K127D).

ADD  
D4